A theoretical model of neural maturation during the spinal cord neurogenesis

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Abstract

Cellular differentiation is a tightly regulated process involving intricate network of interacting signaling and transcription factors. Signaling information provides the extracellular cue interpreted by the transcription factor to drive cellular response, but cross regulation between signaling and transcription factor provides the complexity to the system making it robust and stable. Differential equation based mathematical models provide an important theoretical tool to understand the behavior of these intricate networks. Here we have used differential equation based models to understand the spatiotemporal dynamics of a signal-transcription factor gene networks controlling spinal cord cell maturation. We show that the behavior of dynamic signaling information and downstream transcriptional readout depends upon the strength of interaction among network partners. Our results supports the idea that signaling factors provides spatio-temporal cues to transcription network that ultimately determines the response. Finally, our results outline the possible interaction conditions that could explain observed *in vivo* development patterns.

Introduction

Cellular differentiation entails sequential transition in cellular state from high potency stem cell to low-potency specialized cell, guided by the intercellular signaling regulating the activity of underlying transcription factors. Signals provide spatio-temporal information to the decision making transcription factor network active in the cell (Basson, 2012; Egli et al., 2008; Housden and Perrimon, 2014; Perrimon et al., 2012). In turn, transcriptional network dictates cell's competence and response to the extracellular signaling (Halfon et al., 2000; Zaret and Carroll, 2011; Zaret et al., 2008). Response to signaling information in turn changes the transcriptional components thus creating an intricate dynamic cross-regulatory system responsible for a cell's differentiation down the lineage (Basson, 2012; Housden and Perrimon, 2014; Perrimon et al., 2012).

Vertebrate spinal cord provides an advantageous model to study the cross-regulatory dynamics involved in central nervous system development in particular, and differentiation in general. The head (rostral) to tail (caudal) development of spinal cord during vertebrate body extension results into a characteristic spatial separation of temporal differentiation events (Gouti et al., 2015; Henrique et al., 2015; Wilson et al., 2009), facilitating the study of the regulation of stage specific events. Briefly, the spinal cord neural progenitors (NPs) are derived from bipotent neuro-mesodermal progenitor (NMP) population; that also has the potency to give rise to mesodermal precursors. NMP are located at the caudal most end of the neural tube in a region termed as caudal lateral epiblast and node streak border, in early embryo, and in caudal neural hinge at later stages (Gouti et al., 2015; Henrique et al., 2015; Wilson et al., 2009). During NP maturation, cells exit of the NMP domain and are guided by a GRN that drives their sequential

cell state transition as they become part of the elongating spinal cord (Gouti et al., 2014; Henrique et al., 2015).

NP maturation is driven by the antagonistic FGF/WNT-RA activities turning on or off key transcription factors as the cell undergoes caudal-to-rostral maturation events (**Fig 1A**). In the caudal to rostral direction, FGF/WNT-RA gradients drive the expression of bipotency markers *T/Bra*, *Sox2*, and *Nkx1.2* characteristic of NMP , then the loss of *T/Bra* in early NP, followed by the loss of *Nkx1.2* and the induction of *Pax6* in late NP (ref), and finally the transcription of NP mauration marker *Ngn2* (**Fig 1B**) (Diez del Corral and Storey, 2004; Gouti et al., 2015). These transcription factors are essential for the identity and function of the cell as it undergoes progressive maturation. Until recently, however, little is known about the translation of the signaling information at the transcriptional factor level that results into proper spatio-temporal segregation of cellular states. We recently showed that CDX4 is at the core of the GRN that regulate the sequential maturation of NP into mature neuronal precursors (**Fig 1C,**(Joshi et al., 2019)).

Here we dynamically analyze the GRN driving NP maturation using differential equation. As, the transcription factor depend upon the overlying FGF-WNT-RA interactions, we first analyzed the effectives of this signaling network to work as a signaling switch as postulated by Olivera and Storey (Olivera-Martinez and Storey, 2007). Then we used the signaling dynamics as the input to evaluate the performance of the underlying transcription network in its ability to generate pattern of wild type cellular states as observed in wild type. Our results suggests that interaction strength among network partners is the dominant predictor of the response of the GRN. Our results show that signaling interaction can give rise to various developmentally observed phenotypes based on a subset of interaction parameters, and these behaviors are stable to minute changes in these

parameters. Our results also suggest that strength of transcriptional activation/repression can modulate the response of GRN to a given overlying information. Further, due to intensive cross-regulation the system is robust to noisy signal. Finally, we outline conditions that could explain the *in vivo* observed behavior of the postulated GRN during NP maturation.

Methods

Transcriptional regulation of genes in the interaction network were modeled by differential equations describing the rate of change of mRNA and protein (Santillan, 2008; Sherman and Cohen, 2012; Shi et al., 2017), as follows

mRNA dynamics:

$$\frac{dM}{dt} = \alpha_m H_1 - \beta_m M H_2$$

Protein dynamics:

$$\frac{dP}{dt} = \alpha_p M H_3 - \beta_p P H_4$$

,where,

α_m = Transcription rate constant. β_m= mRNA decay rate constant. α_p= Translational rate constant. β_p= protein decay rate constant.

M and *P* are mRNA and protein concentrations, respectively.

H_1 , H_2, H_3, H_4; are Hill functions describing regulatory interaction by upstream factors.

Hill function describing activation is of the form:

$$H_i = \frac{\left(\frac{A}{A_c}\right)^n}{1 + \left(\frac{A}{A_c}\right)^n}$$

,where A is the concentration of activator protein, A_c is the Hill constant and n is the Hill coefficient of cooperativity.

Multiple activators can be described with the equation

$$H_i = \frac{\left(\frac{A1}{A1_c}\right)^{n1} + \left(\frac{A2}{A2_c}\right)^{n2}}{1 + \left(\frac{A1}{A1_c}\right)^{n1} + \left(\frac{A2}{A2_c}\right)^{n2}}$$

The Hill function describing repression is of the form:

$$H_i = \frac{1}{1 + \left(\frac{R}{R_c}\right)^m}$$

,where R is the concentration of repressor protein, R_c is the Hill constant and m is the Hill coefficient of cooperativity.

Multiple repressors can be described with the equation

$$H_i = \frac{1}{1 + \left(\frac{R1}{R1_c}\right)^{m1} + \left(\frac{R2}{R2_c}\right)^{m2}}$$

Finally, a regulation where both an activator and a repressor act together can be represented with the equation:

AND configuration: Where activator and repressor can bind on separate regulatory regions.

$$H_i = \frac{\left(\frac{A}{A_c}\right)^n}{1+\left(\frac{A}{A_c}\right)^n} \; x \; \frac{1}{1+\left(\frac{R}{R_c}\right)^m}$$

OR configuration (Competitive inhibition): Where activator and repressor competes for a regulatory region.

$$H_i = \frac{\left(\frac{A}{A_c}\right)^n}{1+\left(\frac{A}{A_c}\right)^n+\left(\frac{R}{R_c}\right)^m}$$

In case of a diffusing molecule, a diffusion term, $\mu \frac{d}{dx^2}$,was added on the left side of the equation (μ is diffusivity coefficient, *x* is the spatial dimension).

Differential equations representing the interaction network were numerically solved using MATLAB solver. Partial differential equations showing interactions among factor with diffusing molecules were solved using MATLAB pdepe solver. Ordinary differential equations were solved using MATLAB ode45 solver. MATLAB was also used to plot the simulations of the equation systems.

In situ hybridization

Analysis of gene transcription by *in situ* hybridization was done using digoxigenin (DIG)-labeled antisense RNA probes synthesized and hybridized using standard protocol (Wilkinson and Nieto, 1993). Briefly, embryos were harvested at the appropriate stage and fixed with 4% paraformaldehyde diluted in 1x PBS at 4° C overnight, before processing for *in situ* hybridization. After a series of washes, embryos were exposed overnight in hybridization

solution to DIG-labeled antisense RNA probes against *Pax6*, *Nkx1.2*, *Bra*, *Sox2*, *Cdx4 or Ngn2*. mRNA expression was detected using an Alkaline Phosphatase coupled Anti-DIG antibody (Roche) and developing embryos with nitro-blue tetrazolium salt (NBT, Thermo Scientific) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP, Biosynth) at room temperature until dark purple precipitate deposited revealing the areas of gene transcription. Post-development, embryos were washed with 1x TBST and then fixed in 4% PFA.

Results and discussion

FGF-WNT-RA signaling interaction network could drive signaling switch under specific conditions

For the objective of simulating signaling dynamics, we based our model on previously experimentally defined regulatory interactions between FGF, WNT and RA signaling factors (Diez del Corral et al., 2003; Olivera-Martinez and Storey, 2007). As several FGF and WNT factors are transcribed within and around the caudal neural plate performing redundant function (Lunn et al., 2007; Olivera-Martinez and Storey, 2007), for simplicity we focused on the factors shown to have the most influence on the system: FGF8 and WNT8C (Olivera-Martinez and Storey, 2007). *Fgf8* is transcribed in the caudal stem zone (**Fig 1A**), where it inhibits *Raldh2* transcription (no RA production), and promotes *Cyp26a* transcription (high RA degradation) (Diez del Corral and Storey, 2004). In the caudal stem and transition zone, FGF8 also activates *Wnt8c* transcription (Olivera-Martinez and Storey, 2007). WNT8C then induces *Raldh2* transcription in the region where activation outcompetes FGF8-mediated repression (Olivera-

Martinez and Storey, 2007). RA produced by RALDH2 activity diffuses into the caudal spinal cord from *Raldh2* expressing somites where it inhibits *Fgf8* transcription (Diez del Corral and Storey, 2004; Kumar and Duester, 2014). Together these interactions give rise to an extended negative feedback loop (**Fig 2A**). Our model also postulates a positive autoregulation of *Raldh2* by RA, as in developing embryos once activated *Raldh2* expression is maintained in the somites even in the absence of inducing WNT8C signal.

Cell proliferation in the stem zone drives the caudal-ward extension of the vertebrate axis resulting in differentiated tissues at the rostral end (Henrique et al., 2015). The signaling interaction were simulated in a caudally moving spatial domain of constant length extending rostrally from the stem cell zone, thus appearing stationary from the perspective of caudal end (**Fig 2B**). We used partial differentiation equations to simulate the modelling interactions as diffusing signaling factor were involved. As each parameter including rate constants of synthesis/degradation, diffusion and interactions strengths could potentially impact system behavior; for the purpose of our study we focused on varying the interaction parameter, namely, Hill constant; keeping rest of the parameters unchanged. Hill constant in the Hill equation is the concentration of a factor at which the rate of reaction, regulated by the factor, is half of the maximum possible rate. Hence, Hill constant can act as the measure of interaction strength (**Supplemental information, Fig S1**).

The model simulates how the signaling interaction system will behave with a given initial exponential *Fgf8* transcript gradient in a spatial domain, generated as a result of the caudal movement of the stem zone which is the source of *Fgf8* transcription. Starting with the same initial exponential *Fgf8* and its inducer concentration and varying the interaction strength between FGF, WNT and RA we obtained various temporal signaling information profiles that

can be grouped into four broad behaviors: FGF-dominant, FGF-RA balance, FGF-RA switch, and aberrant.

FGF8 dominance: Interaction conditions where FGF8 dependent repression of *Raldh2* transcription outweighs RA dependent repression of *Fgf8* transcription (such as combination I, **Table 1**), the system doesn't show appreciable *Raldh2* transcription and RA production (**Fig 2 C, Fig S2**). Such a system would lead to maintenance of pluripotent stem progenitor cells without differentiation.

Table 1. Hill constant values for various observed signaling factor dynamics

Hill constants	I	II	III	IV	V	VI
F_{FW}	10	10	10	10	10	10
F_{FR1}	1	1	5	10	10	10
F_{FR2}	2	10	15	10	10	10
W_{WR}	1	1	0.2	0.5	0.2	0.5
R_{RF}	10	1	0.2	0.1	0.3	0.45
R_{RR}	50	50	50	50	50	50

FGF8-RA balance: Under appropriate interaction conditions; FGF8, WNT8C and RA signaling domains settle to a stable steady state profile after initial dynamics (**II, Table 1; and Fig 2D, Fig S2A, B**). Such steady state is achieved when the activating and repressive interactions of the system have reached an equilibrium, and if other conditions don't change (such as: speed of caudal movement), then the differentiation front, as defined by the region of overlap of FGF8

and RA (Goldbeter et al., 2007), will continue to move caudally along with the stem zone at the same speed.

FGF-RA switch: The most interesting behavior obtained from simulation is where the system starts with an initial *Fgf8* mRNA gradient and ends with high, broad RA distribution (**III, Table 1; and Fig 2E, Fig S1A**). This behavior simulates the possibility where the system starts with a stem cell zone and as time progress the differentiation front move closer and closer to the stem cell zone finally overtaking the stem zone. As the differentiation front moves closer to the caudal end, the size of the stem cell zone decreases progressively and in the end a fully differentiated region is left. Importantly, this is the mechanism by which axial elongation is thought to cease in embryos (Cunningham et al., 2016; Olivera-Martinez et al., 2012). The speed of differentiation also depends on interaction that in turn could possibly determine the axial length (**IV, V, VI**; **Fig 3A, B, and C**).

Aberrant behaviors: In addition to above described behavior, the FGF-WNT-RA interaction system also resulted in certain 'unnatural' profiles that we termed as *aberrant behaviors*. Especially in the absence of strong RA feedback on Raldh2 production, we observed a peak of RA signaling abutted by RA deficient region on either side or temporal oscillation in the size of FGF-RA domains (**Table S1 VII, VIII**; **Fig S3**).

Altogether, our results show that the FGF8-WNT8C-RA interaction network as postulated by Olivera-Martinez et al. (Olivera-Martinez and Storey, 2007) could indeed give rise a signaling switch that could travel caudally with the caudal elongation of embryonic axis. However, the behavior of the switch depends on the interaction parameters, which regulates the position and the speed of the differentiation font.

Transcription factor interactions dictates the transcription factor domains driven by the signaling switch

For the objective of simulating transcription factor dynamics, FGF-RA balance profile was chosen as the representative signaling input for the downstream transcription network. The simulation focused on the transcriptional profile of a cell that is located in the stem cell zone at the start of simulation (t=0). As the time proceeds, this cell exits out of the stem zone and progressively matures (**Fig 4A**). For simplicity, the cell is assumed not to undergo any cell division during its maturation from NMP state to the neurogenic state. As the NMP domain and associated signaling domain moves caudally, this cell is stationary, however, from the point of view of the NMP domain it appears to move rostrally, thus experiencing a switch from high FGF-low RA to low FGF-high RA signaling profile (**Fig 4B**). Additionally, from the point of view of the cell the spatial signaling information is perceived temporal information. The temporal change in signaling information drives temporal changes in transcription factors expression. Cells exiting out the NMP domain go through the same changes in the transcription factor expression as they are under the same signaling input, for the case of FGF-RA balance profile. As these cells are arranged spatially in order of their birth, from the caudal NMPs to the rostral differentiated cells, the temporal changes in transcription factors give rise to spatial changes in profiles.

Previously our and other groups have described the transcription factor network regulating graduation maturation of NMPs into neurogenic cells (**summarized in Fig 4C**). We simulated the expression domain of these markers to study the role of transcription factor interactions strengths in properly regulating the maturation events. We also included two putative factors, *X* and *Y*, for CDX4 and PAX6 dependent indirect repression (Joshi et al., 2019).

FGF8 is involved in activating genes including *T (Bra)* and *Cdx4* at the caudal end (Bel-Vialar et al., 2002; Boulet and Capecchi, 2012). Wnt8c is involved in the activation of *Nkx1.2* (Tamashiro et al., 2012) and *Cdx4* (Nordstrom et al., 2006). *Nkx1.2* in turn inhibit itself (Tamashiro et al., 2012) and is indirectly inhibited by Cdx4 (Joshi et al., 2019). *Pax6* is activated by CDX4, and that activation depends on presence of RA (Joshi et al., 2019). RA also independently activate *Pax6* (Novitch et al., 2003). In addition, NKX1.2 inhibits *Pax6.* PAX6 is the sole activator *Ngn2* (Bel-Vialar et al., 2007), which is inhibited indirectly by CDX4 (Joshi et al., 2019). *X* is a putative repressor downstream of CDX4, whose expression is assumed to be negatively regulated by FGF8; that is responsible for CDX4 dependent repression of *Nkx1.2* in the early NP. Similarly, *Y* is a putative repressor downstream of PAX6, also assumed to be inhibited by FGF8, that is responsible for PAX6 dependent inhibition of *Cdx4* in late NP (Joshi et al., 2019).

An overview of the interactions suggests that the spatial dynamics of the signaling factor network would be sufficient to drive the transcription network and give rise to the correct spatio-temporal generation of fates. As FGF8 concentration is high on the caudal (left) side of spatial domain and RA is high on the rostral (right) side of spatial domain, transcription factors regulated by each should be present only on their respective side. In the middle the cross-repressive interaction should be able to achieve the observed transition in cellular state.

However, if all the interactions in the network are equally moderate (Hill constants =20, **Fig 4D**) or equally strong (Hill constants =2, **Fig 4E**), than the network doesn't result in proper spatial resolution of temporal states seen in wild type embryos. Only a subset of interaction strengths, on such condition set listed in Table2, give rise to correct spatial order of identities (**Fig 4F**).

Table 2: Hill constant for correct spatio-temporal distribution of cellular states.

Hill constants	Description	Value
F_{FT}	FGF8 dependent activation of *T*	10
F_{FS}	FGF8 dependent activation of *Sox2*	50
F_{FC}	FGF8 dependent activation of *Cdx4*	5
F_{FX}	FGF8 dependent repression of *X*	1
F_{FY}	FGF8 dependent repression of *Y*	1
W_{WN}	WNT8C dependent activation of *Nkx1.2*	10
W_{WC}	WNT8C dependent activation of *Cdx4*	10
S_{ST}	SOX2 dependent repression of *T*	20
T_{TS}	T dependent repression of *Sox2*	2
N_{NN}	NKX1.2 dependent repression of *Nkx1.2*	100
N_{NP}	NKX1.2 dependent repression of *Pax6*	20
C_{CX}	CDX4 dependent activation of *X*	10
C_{CP}	CDX4-RA complex dependent activation of *Pax6*	10
R_{RS}	RA dependent activation of *Sox2*	1
R_{RP}	RA dependent activation of *Pax6*	10
X_{XN}	X dependent repression of *Nkx1.2*	1
X_{XN2}	X dependent repression of *Ngn2*	1
P_{PY}	PAX6 dependent activation of *Y*	5
P_{PN2}	PAX6 dependent activation of *Ngn2*	20
Y_{YC}	Y dependent repression of *Cdx4*	5

Signaling factors encode spatio-temporal information read out by the transcription network

To evaluate the individual role of signaling and transcription factors in driving maturation events, we tested for changes in the transcription readout upon disruption in the signaling information or transcription regulations. First, we tested the response of the transcription network to the noise in overlying signaling information. In the simulations, both periodic disturbance (**Fig 5A**) and random noise (**Fig 5B**) were very much tolerated by the transcription network without any distortions in the spatio-temporal resolution of the cellular states. This suggest that transcription network has built in robustness to the extrinsic noise.

Then, we evaluated the role of signaling gradients in determining the spatio-temporal resolution. Replacing the exponential gradient of all the signaling factors with a Boolean switch (**Fig 5C**) or with linear gradients (**Fig 5D**), resulted in loss of proper resolution of transition zone identity. This suggests that the signaling factors encode the spatial information which otherwise is absent in the transcription network. A change in the spatial information will result in different read out by the transcription network.

We previously proposed a central role of CDX4 in regulating proper maturation of NPs in the PNT. To theoretically test the role of CDX4 in the transcription network *in silico*, we evaluated the transcription profile generated by increasing (**Fig 6A**), elimination (**Fig 6B**) and with noise in CDX4 activity (**Fig 6C**). Gain of *Cdx4* downregulated *Nkx1.2* and *Ngn2* transcription, similar to what we have shown *in vivo* (Joshi et al., 2019). Conversely, loss of *Cdx4* leads to overlap in expression domains of *Nkx1.2*, *Pax6* and *Ngn2*. *In ovo* experiments lacked a proper knockdown strategy, and were supplement by overexpression of repressive form of CDX4 rather than a

genetic knockout. Hence, there are discrepancies between the loss of *Cdx4* simulation output and phenotypes obtained from ENRCDX4 overexpression experiments. Both overexpression and loss of *Cdx4* simulations suggest the role of *Cdx4* in proper segregation of transition zone and neural tube identities. We investigated the robustness of the system by introducing noise in the transcription of *Cdx4*, that was observed to be well tolerated by the GRN

Conclusions

Signaling factor simulation recapitulates signaling dynamics observed in natural systems.

Our simulations describe the various behaviors the FGF8-WNT8C-RA system can exhibit under various possible interaction conditions. We propose a model of vertebrate body extension where by modulating interaction strength by the means of transcriptional-epigenetic factors, a combination of described behaviors could regulate spatio-temporal dynamics involved in vertebrate body extension. For example: initially the system could start with FGF dominant system, then switch to FGF-RA balance to continue steady extension of the axis and finally move to FGF-RA switch mode to end any further elongation of the body axis. Indeed the FGF8-RA balance system could explain the elongated axis of some vertebrates, such as snakes, that has numerous vertebrae and a long neural tube form. The third behavior, FGF-RA switch describes a process that involves RA signaling overtaking the FGF signaling in the progenitor/stem cell spatial field causing the cells to differentiate, and eliminating all precursor cells that could elongate the body axis. The time duration of RA takeover could also decide the length of the body axis, as faster RA takeover will lead to shorter body axis and vice versa.

Interaction strengths dictate the interpretation of signaling information.

Strength of cross-regulatory interactions (here Hill constants) are important in determining the output of the signaling information. In other words, systems can use same signaling information to drive different physiological outputs by playing with the interaction strengths of the transcription regulation. As observed in simulations (**Fig 4C, 4D and 4E**), varying the Hill constants resulted into different spatio-temporal dynamics of the transcription network under the same signaling information. The interaction strength is a measure of the threshold of regulatory factor required for transcription factor regulation. A possible way of modulating the threshold is via modulating strength of the enhancer. A weak enhancer requires a greater amount of regulator to be active, hence a higher Hill constant. Conversely, a strong enhancer is active even with low amounts of regulator due to its lower Hill constants. By utilizing enhancers of different strength or by varying the strength of an enhancer, transcription factor expression dynamics could be varied in a natural system (Miyagi et al., 2006; Simmons et al., 2001; Woodcock et al., 2013).

In living systems, one of the ways of modulating strength of an enhancer is by epigenetic regulation (Calo and Wysocka, 2013). Chromatin modification, by masking/unmasking enhancer regions, modulates the ability of transcription factors to bind to regulatory regions (Doyle et al., 2014; Kim et al., 2004; Patel et al., 2013; Plachetka et al., 2008). Also epigenetic modification of histone residue can modulate the activity of enhancers (Stonestrom et al., 2015; Wu et al., 2011; Zhang et al., 2012). Another possible way of regulating the strength of enhancer interaction is the presence of cofactors (McClellan et al., 2013; Merino et al., 2015). In fact, a combination of chromatin and cofactors determines the enhancer activity during development (Fry and Farnham, 1999; Voss and Hager, 2014). Altogether, the data about regulatory strengths could be tested *in vivo* to validate the model and further refine the parameter set.

Transcriptional network recapitulates NMP to neurogenic state transition as seen in caudal neural tube.

Results from our *in silico* simulation reemphasize the role of CDX in coordinating upstream signaling factors and downstream transcription network. In the present model, CDX4 plays a determining role in generation of caudal transition zone (*Nkx1.2+ Pax6- Ngn2-*) and rostral transition zone (*Nkx1.2- Pax6+ Ngn2-*). In the *Cdx4* manipulation simulations (**Fig 6**), *Cdx4* overexpression results in loss of *Nkx1.2* and *Ngn2* suggesting that CDX4 promotes acquisition of rostral transition identity, and inhibits both caudal transition identity and neurogenic identity. As in the present model RA limits CDX4's regulation of *Pax6*, over loading the system with CDX4 didn't expand its caudal domain of expression. Conversely, in the loss of *Cdx4* results in expansion of *Nkx1.2* and *Ngn2* domains rostrally and caudally, respectively; suggesting the role of CDX4 in separating caudal transition zone from neurogenic zone. In these conditions, changes in *Pax6* domain weren't significantly altered, even in the absence of CDX4, because RA was able to activate *Pax6*. However, due to expansion of *Nkx1.2* and *Ngn2* the rostral transition identity was lost. Both gain and loss of *Cdx4* simulations suggest that CDX4 is involved in establishing rostral transition zone (*Nkx1.2-Pax6+Ngn2-*) that separates caudal transition zone (*Nkx1.2+Pax6-Ngn2-*,) from neurogenic zone (*Nkx1.2-Pax6+Ngn2+*), hence regulates the pace of differentiation.

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Figures

Figure 1

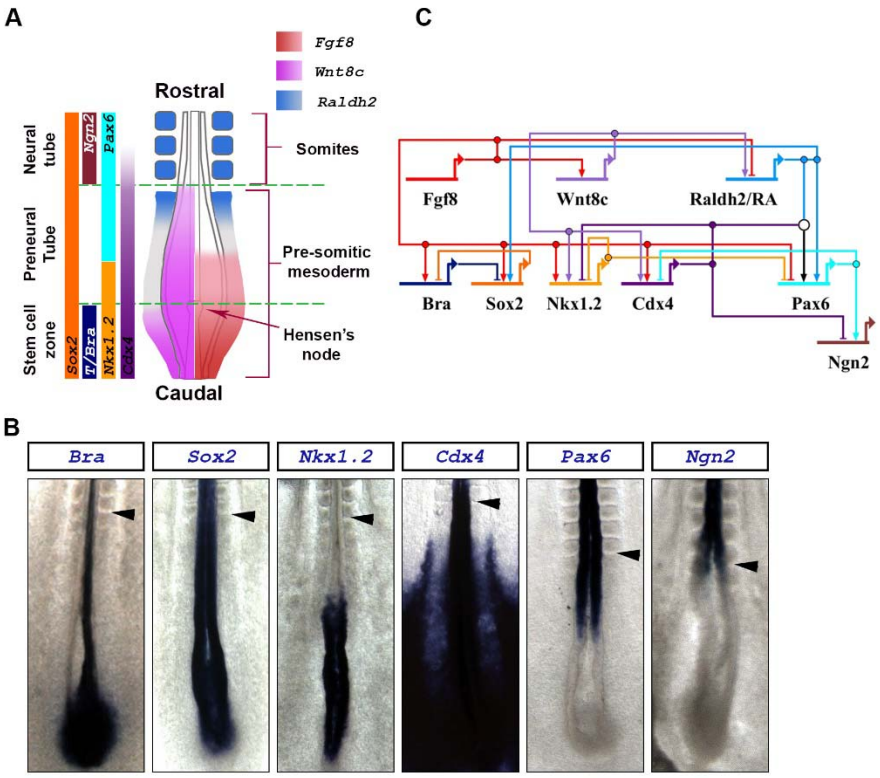


Figure 2

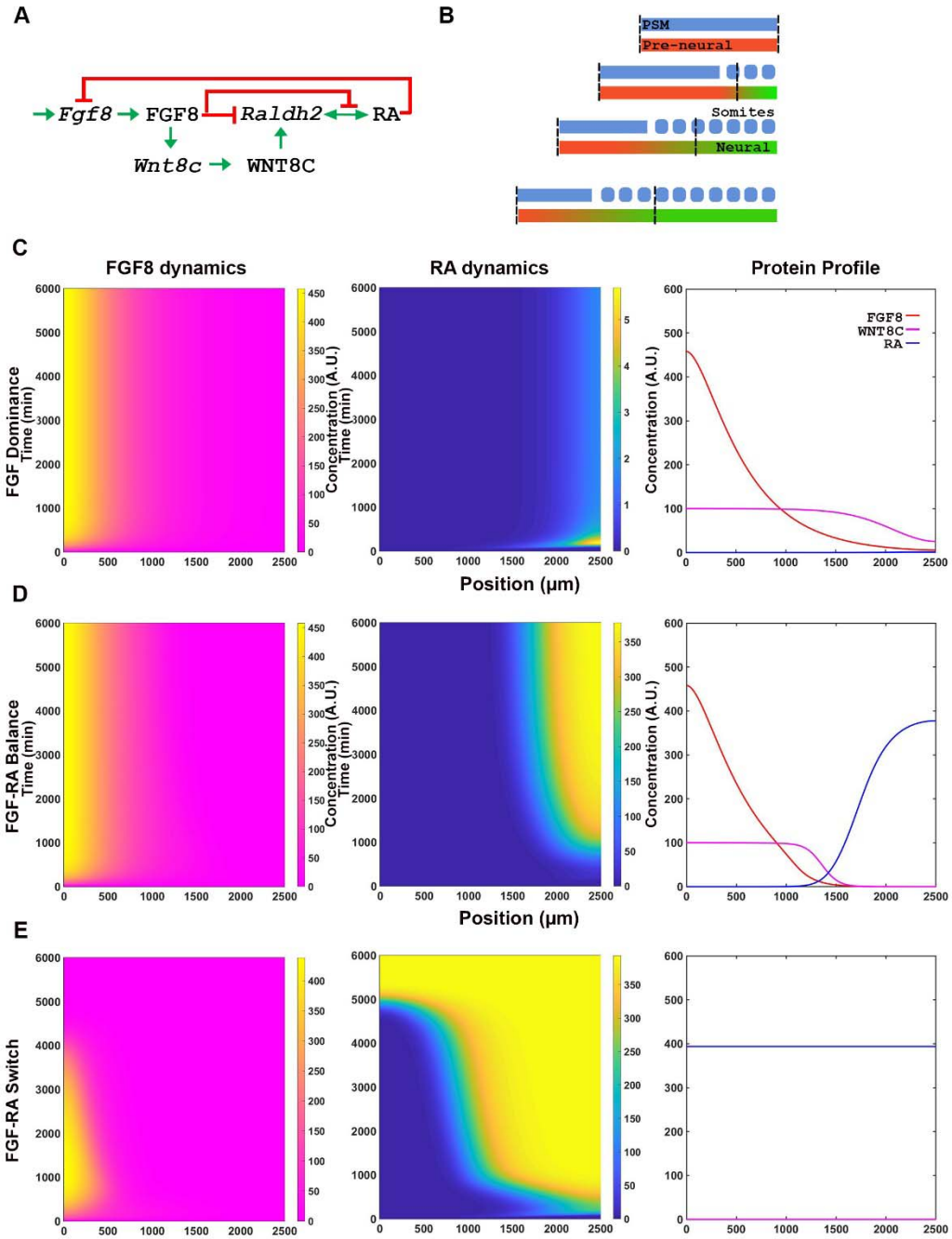
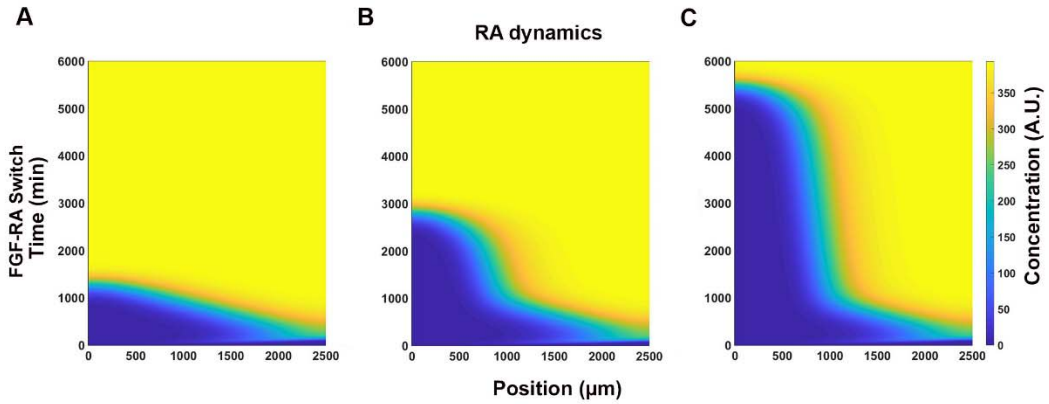


Figure 3



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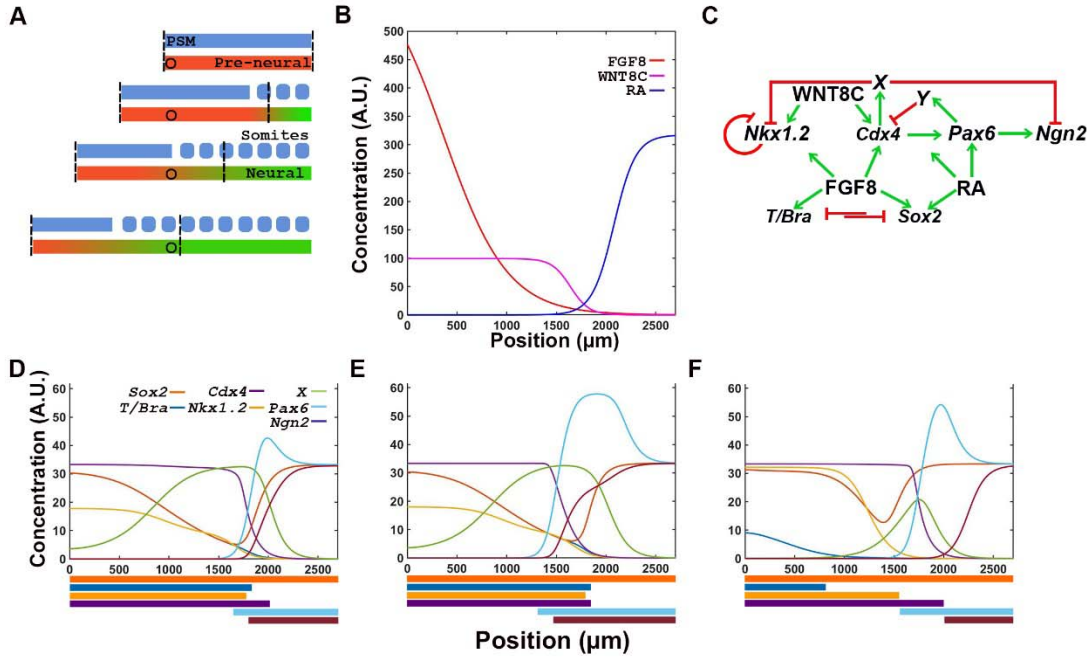

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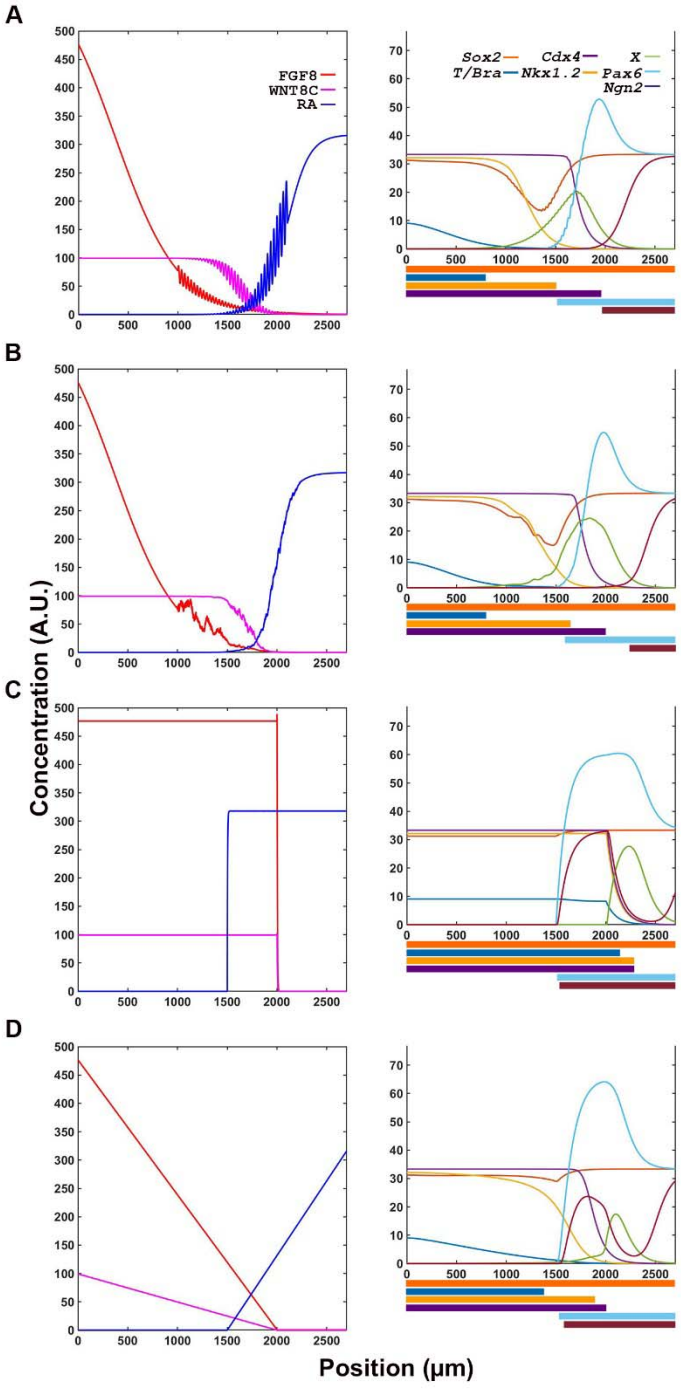

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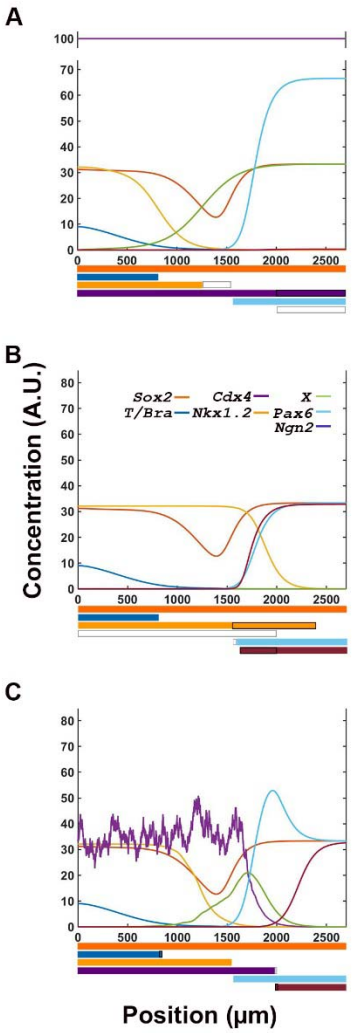

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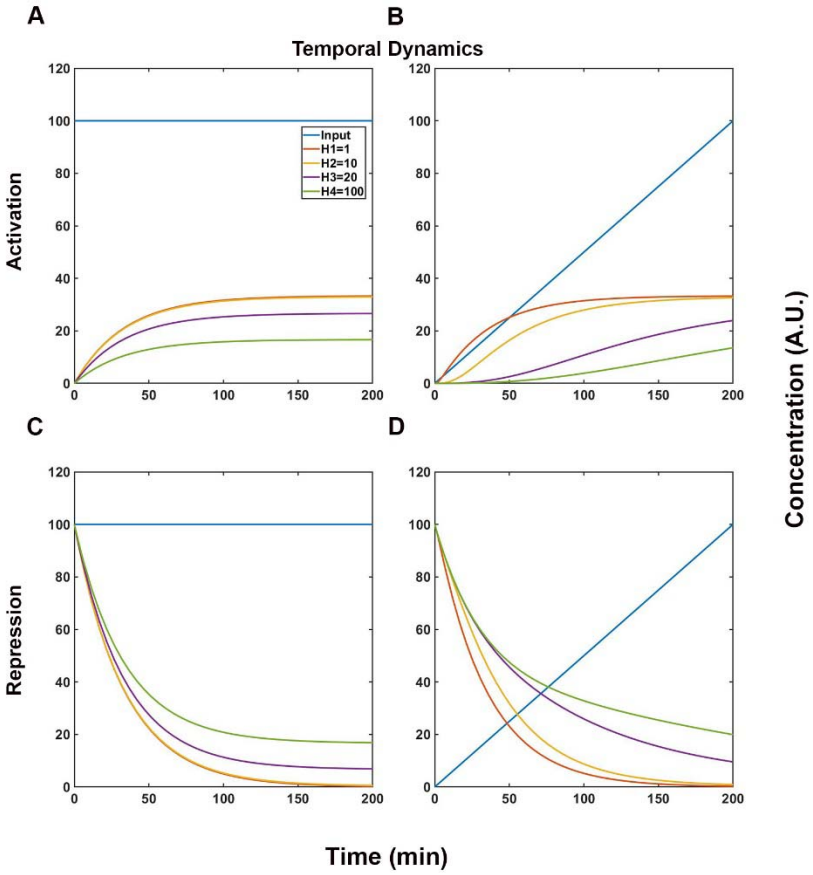
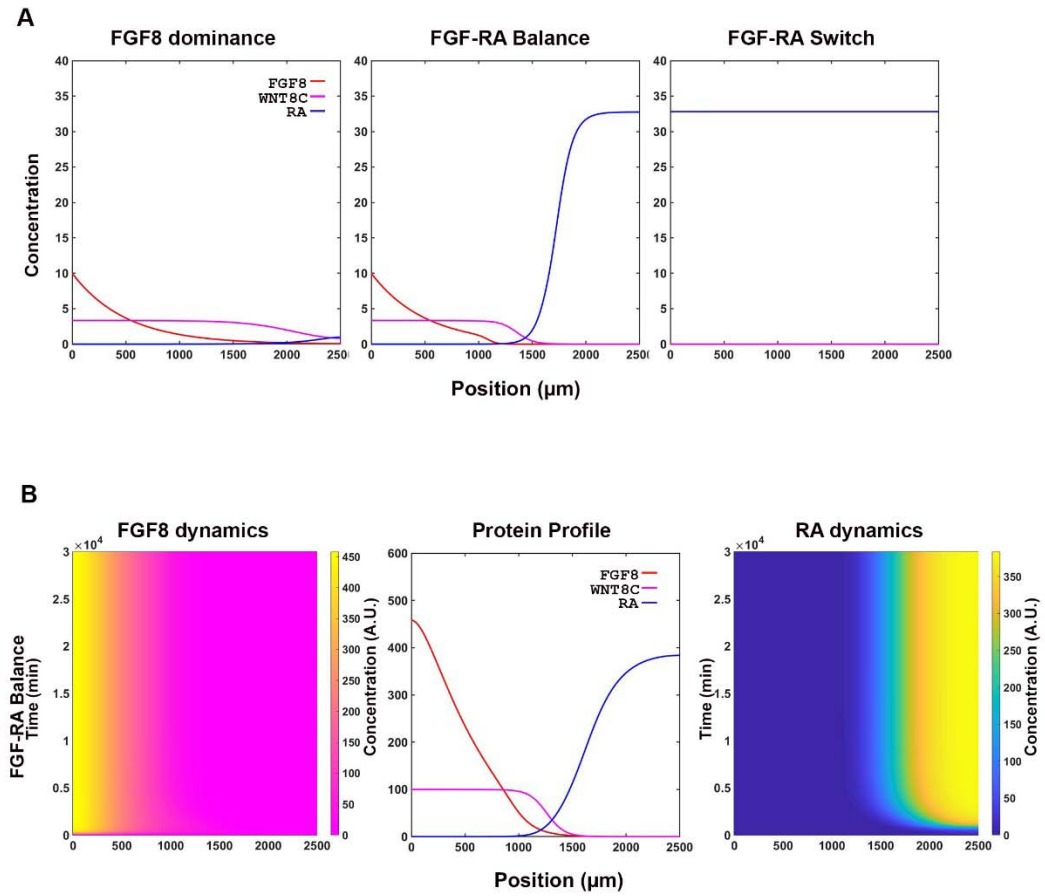


Figure S2



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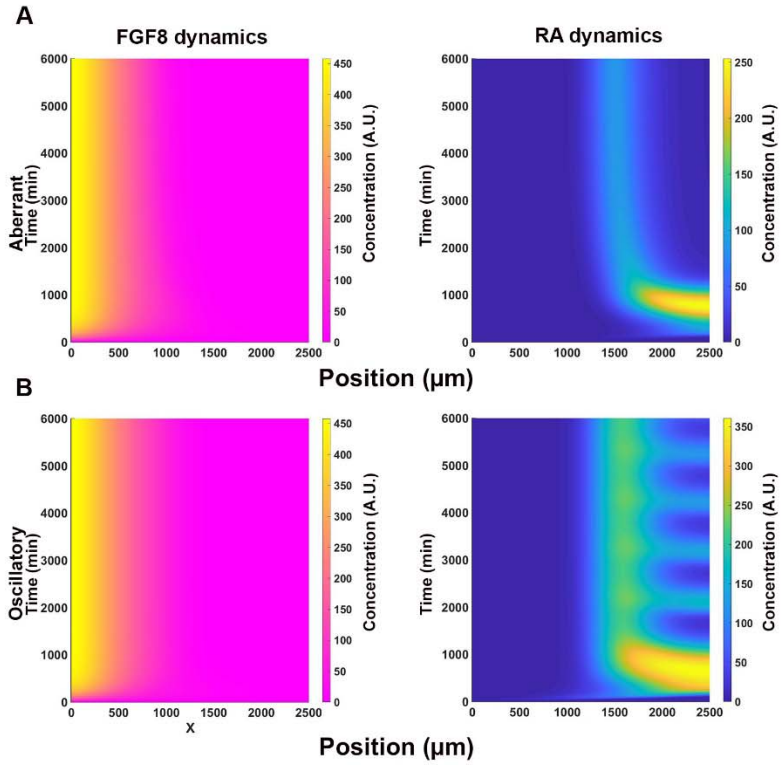

Figure legends

Figure 1. FGF-WNT-RA signaling results in nested transcription factor expression domains in the caudal spinal cord. A) Schematic representing expression domain of *Fgf8*. *Wnt8c* and *Raldh2* in caudal spinal cord and consequent transcription factor domains as bars on left in a stage HH10-11 chicken embryo. B) Expression domains of key transcription factors involved in caudal spinal cord maturation detected via in situ hybridization. C) Postulated gene regulatory network showing interaction between signaling factors and transcription factors.

Figure 2. FGF-WNT-RA signaling interaction could result into various dynamic behaviors. A) Schematic representing interaction among FGF8, WNT8C and RA signaling. B) Schematic showing the spatial domain of constant length moving caudally at a constant rate where signaling interactions were simulated. Initially the entire domain is undifferentiated tissue that later start differentiating at the rostral end with the differentiation domain overtaking the undifferentiated domain proportion. C) Heat-map depicting FGF8 (left) and RA (middle) spatial domains over the duration of simulation in the region of focus. Left panel shows expression domains of FGF8 (red), WNT8C (pink) and RA (purple) at t=6000 min.

Figure 3. Spatio-temporal dynamics FGF-RA switch dictates length of body axis. A, B and C) FGF-RA mutual repression dictates the temporal duration of FGF-RA switch that in turn dictates the length of body axis.

Figure 4. Transcriptional interaction strengths dictates the response to the overlying signaling information. A) Schematic of spatio-temporal domain of simulation of transcription factor interactions and response to signaling input. The circle represent the reference cell that is

exposed to the overlying dynamic signaling information. B) Reference signaling factor input for simulation in D. C) Schematic of interactions between transcription and signaling factors. X and Y are hypothetical candidates responsible for CDX4 and PAX6 dependent indirect repression, respectively. D) Transcription profile of cell with all interactions equally moderate (hill constants=20), E) all interaction equally strong (hill constants=1), and F) interaction defined in Table 2.

Figure 5. Signaling information is important in defining transcription factor domains. Noisy A) oscillatory or B) random fluctuation in signaling information affect transcriptional factor expression domains compared to Fig 4F. Manipulating signaling gradient information from exponential to C) discrete or D) linear also affect transcriptional factor expression domains compared to Fig 4F.

Figure 6. CDX4 regulate transcription factor domains downstream of signaling information. A) Loss of *Cdx4* expression expands *Nkx1.2* and *Ngn2* domains, with small reduction in *Pax6* domain. B) Overexpression of *Cdx4* reduces *Nkx1.2* and *Ngn2* domains. C) Noisy transcription of *Cdx4* has very minute effect on transcription factor domains. Grey empty rectangle depicts loss in spatial expression and black filled rectangle depict gain of expression of particular gene compared to expression domain in Fig 4F.

Supplementary information

Supplementary figure legends

Figure S1. Hill constant determine the strength of activating/repressive interactions. For a constant amount of activator (A), downstream targets with lower Hill constant (H1=1 vs H4=100) have higher rate of production with a higher value of final concentration. In the case of graded input of activator (B), downstream targets with lower Hill constant are activated first. For a constant amount of repressor (C), downstream targets with lower Hill constant have faster decline in concentration with a lower value of final concentration. In the case of graded input of repressor (D), downstream targets with lower Hill constant are repressed first. Blue: Input. Red: Hill constant (H1) =1. Orange: Hill constant (H2) =10. Purple: Hill constant (H3) =20. Green: Hill constant (H4) =100.

Fig S2. FGF-WNT-RA signaling interaction results in different stable mRNA profiles. mRNA profile associated with protein profile at t=6000 min shown in in Fig 2C left panels. B) FGF-RA balance simulation ran for t=30,000 min show the profile it stable for longer simulation time too.

Fig S3. Unnatural RA profile in the absence of positive RA feedback. Weak positive feedback of RA on Raldh2 production results in A) Aberrant RA peak in the middle of spatial field or B) oscillatory RA behavior.